

REMARKS

I. Support for the Amendments to the Specification and the Claims

It has come to Applicant's attention that the amendment to the cross-references paragraph of the specification as filed on April 20, 2010, was inconsistent with the previous amendment to the cross-references paragraph made in the Preliminary Amendment filed concurrently with the U.S. national phase on December 23, 2005. Therefore Applicant withdraws the inconsistent amendment made on April 20, 2010, and requests reversion of the language of the specification to the language used for the cross-references paragraph as amended in the Preliminary Amendment of December 23, 2005.

As noted in the Preliminary Amendment of December 23, 2005, support for the amendment to the specification made in the Preliminary Amendment can be found in the PCT application as filed. This application is a 35 U.S.C. §371 national stage of PCT application PCT/US2004/021637, filed July 6, 2004, which claims the priority benefit of U.S. Provisional Application Serial No. 60/485,509, filed July 6, 2003, and U.S. Provisional Application Serial No. 60/485,607, filed July 7, 2003, the disclosures of all of which are incorporated herein by reference. No new matter is added by virtue of the amendments to the specification.

Claims 54, 58-62, and 67 have been amended, claims 1-4, 6-9, 11-30, 32-41, 55-56, 64-66, and 68-81 have been canceled without prejudice, and new claims 82-91 have been added. Claims 1-4, 6-9, 11-30, 32-41, 55-56, 64-66, and 68-81 have been canceled without prejudice to their pursuit in an appropriate divisional or continuation application. Claims 14-28 and 32-41 were previously withdrawn. Claims 54, 57-62, 67, and 82-91 are currently in the application.

Support for the amendments to claims 54, 58-62, and 67 and for new claims 82-91 can be found in the specification, figures, and claims as originally filed. No new matter has been added by virtue of the amendments to the claims.

Additional support for the amendments to claims 54, 58-62, and 67 and for new claims 82-91 can be found in the language of original claims 1-13 and from page 3, line 19, to page 4, line 6; on page 5, lines 5-13; from page 5, line 23, to page 10, line 11; in the Examples; and in the Figures. Claim 59, which was previously dependent on claim 54, is now dependent on new claim 84.

II. Status of the Claims

Claims 1-28 were previously in the application. The claims were subject to an Election/Restriction Requirement, and claims 1-13 (Group I) were elected with traverse.

Claims 1-13, 29-31, and 54-64 were previously in the application, along with withdrawn claims 14-28.

In the previous amendment, filed April 20, 2010, claims 1, 3-4, 6-9, 11-13, 29-30, 54, 56-67, 60-62, and 64 were amended, claims 5, 10, 31, and 63 were canceled, and new claims 65-81 were added. Claims 14-28 and 32-41 were withdrawn.

Currently, claims 54, 58-62, and 67 have been amended, claims 1-4, 6-9, 11-30, 32-41, 55-56, 64-66, and 68-81 have been canceled without prejudice, and new claims 82-91 have been added. Claims 1-4, 6-9, 11-30, 32-41, 55-56, 64-66, and 68-81 have been canceled without prejudice to their pursuit in an appropriate divisional or continuation application.

Claims 54, 57-62, 67, and 82-91 are currently in the application.

III. The Drawings and the Telephone Interview

Applicant thanks the Examiner for the Telephone Interview on November 18, 2010. Although no agreement was reached, Applicant greatly appreciates the time the Examiner spent in discussing the matter with Applicant's undersigned representative.

In the Office Action, mailed June 30, 2010, the Examiner withdrew the previous acceptance (Office Action, mailed October 20, 2009) of the Drawings. The Examiner has objected to the drawings, because two sets of drawings were submitted when the present U.S. national phase application was filed on December 23, 2005.

On August 30, 2010, Applicant filed a Response & Submission of Drawings. Applicant submitted concurrently therewith one set (six sheets, six figures) of drawings for filing in the above-identified patent application. Pursuant to 37 C.F.R. 1.121(d), these drawings were marked "Replacement Drawings" in the upper right margin. Applicant kindly requested substitution of the drawings submitted on August 30, 2010, for the drawings submitted with the originally filed U.S. national phase application.

On August 30, 2010, Applicant also submitted therewith one set (six sheets, six figures) of drawings with the changes marked in red, namely, the removal of the PCT headers. These drawings were in PCT Application PCT/US2004/021637, of which the present application is the U.S. national phase. These figures were also filed with the present U.S. national phase application on December 23, 2005. No new matter has been added to the Figures.

The Examiner had objected to the drawings, because two sets of drawings were submitted. The other set of drawings (four sheets, six figures) had primarily cosmetic changes, e.g., placing the text in all capitals, changing fonts, using a lighter dappling of the bar graphs in Figures 3-6 to enable the lines of Figures 3-5 to have a greater contrast, and changing the orientation of Figures 2-6 from landscape to portrait.

Applicant respectfully requests reconsideration of the Drawings in accordance with the Response & Submission of Drawings on August 30, 2010.

IV. The Rejection of Claims 1-13, 29-31, and 54-64 under 35 U.S.C. §112, Second Paragraph, is Traversed in Part and Rendered Moot in Part, but Accommodated in Part

The Examiner has rejected claims 1-4, 6-9, 11-13, 29-30, 54-62, and 64-81 under 35 U.S.C. §112, second paragraph, for allegedly failing to particularly point out and distinctly claim the subject matter. This rejection is traversed in part, rendered moot in part, and accommodated in part.

Claims 1-4, 6-9, 11-13, 29-30, 55-56, 64-66, and 68-81 have been canceled without prejudice to their pursuit in an appropriate continuation or divisional application, and the rejection of these claims is thereby rendered moot.

A. Independent claims 1 and 54 and dependent claims 2-4, 6-9, 13, 29, 30, 55-62, and 64-67

The Patent Office finally rejected independent claims 1 and 54, because each step allegedly does not have a clear relationship to the one preceding it (Final Office Action, pp. 3-4). Applicant had previously amended these claims so that, e.g., step (b) recites the cells of step (a).

In reply, the Patent Office states:

For example, step (b) as amended in each claim refers to “the cells of step (a),” which could reasonably refer to the purified BMSCs or to the BMSCs after they have been synchronized. [Final Office Action, p. 4.]

Moreover, the Patent Office alleges that the working Examples in the specification, which describe actual reduction to practice, have somehow “further clouded” the issue (Final

Office Action, p. 4), because both step (a) and step (b) involve incubation with growth factors and cytokines.

The Patent Office also rejected dependent claims 2-4, 6-9, 13, 29, 30, 65, and 66 for alleged failure to clarify underlying independent claim 1 and dependent claims 55-62, 64, and 67 for alleged failure to clarify underlying independent claim 54 (Final Office Action, p. 5).

Claims 1-4, 6-9, 13, 29-30, 55-56, and 64-66 have been canceled without prejudice, and the rejection of these claims is thereby rendered moot.

Applicant respectfully disagrees with the Patent Office's position, because one of ordinary skill in the art would understand a recitation specifying the use of the cells of the preceding step, taken in combination with the disclosure of the specification (especially where there is an actual reduction to practice), as an indication of sequentially performed steps.

Nevertheless, Applicant has amended remaining independent claim 54 to include language specifying the order of the steps.

Claims 55-58, 60-62 and 67 are dependent on independent claim 54, and the amendments to claim 54 also apply to these claims. Claim 59 has been amended to be dependent on new claim 84 as an underlying claim.

In the event that this issue is the only remaining issue in the prosecution of the application, however, Applicant would be willing to work with the Patent Office to amend the claims to place them in condition for allowance.

B. Independent claim 68 and dependent claims 11-12 and 69-73

In contrast to the rejection of claims 1 and 54, the Patent Office finally rejected independent claim 68, because the reference in step (b) to “the synchronous cells of step (a)” allegedly lacks antecedent basis (Final Office Action, p. 4), because step (a) does not recite “the synchronous cells” and because step (a) “only requires that synchronous progression through the cell cycle be ‘promoted,’ not that it actually occurs” (Final Office Action, p. 4; *but see* Manual of Patent Examining Procedure [hereinafter “MPEP”] §2173.05(e)).

The Patent Office also rejected dependent claims 11, 12, and 69-73 for alleged failure to clarify underlying independent claim 68 (Final Office Action, p. 5).

Applicant respectfully submits that the scope of claim 68 could be reasonably ascertained by one of ordinary skill in the art, e.g., by observation of the cells, such as taught in the specification or as known in the art.

However, claims 11-12 and 68-73 have been canceled without prejudice, and the rejection of these claims is thereby rendered moot.

C. Independent claims 74 and 78 and dependent claims 75-77 and 79-81

Also in contrast to the rejection of claims 1 and 54, the Patent Office finally rejected independent claims 74 and 78, because “both include extensive function language in step (b), i.e., the limitations beginning with ‘wherein’” (Final Office Action, pp. 4-5). The Patent Office alleges, in pertinent part:

Here, step (b) in both claims 74 and 78 is indefinite in that it requires a “specific” phase of the cell cycle without providing criteria for the selection of that phase other than the circular requirement that it “promote” a “specific” differentiation pathway. The claim defines the time point in step (b) wholly in terms of some undefined outcome that may or may not come to pass (as discussed above, “promote” is not synonymous with “yield”). Step (b) makes an effort to define the method solely in

terms of its outcome, which is indefinite because the metes and bounds of the steps are not clearly defined. [Final Office Action, p. 5.]

The Patent Office also rejected dependent claims 75-77 for alleged failure to clarify underlying independent claim 74 and dependent claims 79-81 for alleged failure to clarify underlying independent claim 78 (Final Office Action, p. 5).

Applicant respectfully disagrees with the Patent Office's position, because one of ordinary skill in the art would understand that the claims are directed to cell cycle phase-specific differentiation of bone marrow stem cells, as supported by the specification.

However, claims 74-81 have been canceled without prejudice, and the rejection of these claims is thereby rendered moot.

D. Claims 29, 61, 70, 76, and 80

The Patent Office finally rejected dependent claims 29, 61, 70, 76, and 80, alleging that the term "differentiation hotspot" is "not adequately defined in the specification, nor is it a term of art" (Final Office Action, p. 6). The Patent Office alleges that "Applicant has not addressed this issue by amendment or particular comment" (Final Office Action, p. 6), but Applicant did address the Patent Office's concerns in the Amendment (mailed April 20, 2010) in the context of addressing the Patent Office's remarks concerning the "reversible differentiation hotspot" (also discussed below), especially given the fact that Applicant had amended claim 29 to include the definition of "differentiation hotspot" provided in the specification. (Applicant also discussed support in the specification for the term "hotspot" with respect to cell cycle [see, e.g., p. 20 of Amendment, mailed April 20, 2010].)

The Patent Office alleges, in pertinent part:

At page 5, lines 23-28, the specification indicates that “differentiation hotspots” are “points where a specific differentiation pathway is favored,” but it is not clear from this definition whether the hotspot is a set point in the cell cycle or whether it varies for each pathway and in what manner. Clarification is required. [Final Office Action, p. 6.]

Claims 29, 70, 76, and 80 have been canceled without prejudice, and the rejection of these claims is thereby rendered moot.

With respect to remaining independent claim 54 and remaining dependent claim 61, Applicant traverses this rejection. First, it is unclear what the Patent Office means by “whether it varies for each pathway and in what manner,” because claim 54 on which claim 61 is dependent is already limited to the treatment of bone marrow stem cells to provide differentiated hematopoietic cells. (Notwithstanding these limitations, Applicant is not required to describe every possible pathway for every species [see, e.g., p. 6, ll. 1-16, which envisions the use of bone marrow stem cells from different species]. One of ordinary skill in the art would recognize that the cell cycles of different species might vary, but that the teachings of the specification could be adapted without undue experimentation.)

Second, the Patent Office has failed to consider the disclosure of the specification in its entirety. For example, the specification provides examples of “hotspots” and “hotspot cells,” which further clarify the claim limitations:

In an exemplary embodiment, the methods of the invention (when applied to murine cells) comprise the culture of unseparated or purified LRH marrow stem cells (or unseparated marrow). Specifically LRH cells are cultured in DMEM with 15% fetal calf sera and steel factor (50 ng/ml), Flt-3 (100 ng/ml), and thrombopoietin in Teflon bottle cultures at 37°C, 5% CO₂ [sic]. Under these conditions, primitive stem cells progress through cell cycle in a highly synchronous fashion. These cells are then harvested at about 32 hours (mid S-phase) or 40 hours (late S-phase), washed, and subcultured in DMEM, 15% fetal calf sera, and GM-CSF, G-CSF, and steel factor (50 ng/ml) and differentiated cell production evaluated out to 14 days of subculture. See Figure 1 for a schematic representation. When 32 hour primary cultured cells are resubcultured, there is a marked increase in megakaryocyte production, while when 40 hour primary cultured cells are resubcultured, a marked increase in granulocytes is seen. These primary time points are referred to herein as

megakaryocyte and granulocyte “hotspots”, respectively, and the *differentiated hematopoietic cells* resulting from these subcultures are referred to herein as “32 hour hotspot cells” and “40 hour hotspot cells”, respectively. [Specification, p. 6, l. 26, to p. 7, l. 8; all emphasis added.]

Therefore, Applicant respectfully disagrees with the Patent Office’s position, because taken in the context of this passage and other passages in the specification (including the Examples), Applicant submits that one of ordinary skill in the art would readily comprehend the claim language of “differentiation hotspot.”

In the event that this issue is the only remaining issue in the prosecution of the application, however, Applicant would be willing to work with the Patent Office to amend the claims to place them in condition for allowance.

E. Claims 30, 62, 71, 77, and 81

The Patent Office finally rejected dependent claims 30, 62, 71, 77, and 81, alleging that the term “reversible differentiation hotspot” is “confusing because it is not clear whether the hotspot is reversible (i.e., sometimes it is a hotspot, sometimes it is not) or whether the differentiation itself is reversible” (Final Office Action, pp. 6-7). In addition to the discussion with respect to “differentiation hotspot” *supra*, this point was addressed at length in the Amendment mailed April 20, 2010, in the Amendment mailed June 12, 2009, and in the Declaration of Dr. Peter J. Quesenberry Pursuant to 37 C.F.R. 1.132 mailed June 12, 2009 (hereinafter the “Quesenberry Declaration”).

Claims 30, 71, 77, and 81 have been canceled without prejudice, and the rejection of these claims is thereby rendered moot.

With respect to remaining claim 62, which has been amended, Applicant respectfully submits that cellular de-differentiation is a concept known and understood for many years by those of ordinary skill in the art. For example, the specification states:

Previous work has shown shifts in murine marrow stem cell engraftment phenotype tied to cell cycle phase. These initial studies were carried out on both unseparated murine marrow and on highly purified LRH marrow stem cells. Critical aspects of these observations were that they occurred **in the first cytokine induced cell cycle transit before cell division, and that they were reversible**. There were also marked shifts observed in 7-factor responsive progenitors **in the first cell cycle which were also reversible**. Of great interest were observations linking an increase in progenitors to a decrease in engraftable stem cells; a so-called progenitor/stem cell inversion. **These too were reversible**. These latter observations were made on unseparated marrow cells. [Specification, p. 5, ll. 14-22; all emphasis added.]

Following the discussions of “differentiation hotspot” quoted above (e.g., p. 5, ll. 23-28; and p. 6, l. 26, to p. 7, l. 8), the specification further states:

Marked variations in differentiated cell production were seen with the first cell cycle transit. Megakaryocyte differentiation was amplified from 3.5×10^3 cells at G_0 to 9.1×10^4 cells in mid-S phase (Figure 5) [previously defined as a 32-hour differentiation hotspot]. Proliferative granulocyte differentiation was amplified from 6.0×10^4 cells at G_0 to 2.4×10^5 cells in mid-S phase (Figure 3 [previously defined as a 32-hour differentiation hotspot]). Non-proliferating granulocyte differentiation was amplified from 8.2×10^4 cells at G_0 to 4.6×10^5 cells in late-S phase (Figure 4) [previously defined as a 40-hour differentiation hotspot]. These data further support a flexible system for hematopoietic regulation in which multiple different outcomes could occur dependent on cell cycle phase and specific microenvironmental influences. Early primitive marrow stem/progenitor cells represent a continuum of reversible phenotypic shifts as opposed to a hierarchy, with continuous change in a reversible fashion. [Specification, p. 10, ll. 20-29; all emphasis added; all bracketed material added.]

Moreover, one of ordinary skill in the art would understand “a differentiated cell” to refer to one of the “differentiated hematopoietic cells” of underlying claim 54 and “a stem cell” to refer to one of the “bone marrow stem cells” of underlying claim 54.

With respect to the Patent Office's remarks concerning the Quesenberry Declaration, Dr. Quesenberry addressed this matter in the context of the "continuum" theory of differentiation, as opposed to the "hierarchical" theory of differentiation previously accepted in the art:

Unexpectedly, we have found that bone marrow stem cells continually change phenotype in a *reversible* fashion tied to cell cycle transit (the "continuum theory"). The present application addresses a portion of this concept, namely, the ability to cause cells to differentiate into various cell types linked to one phase of the cell cycle vs. another phase of the cell cycle, which provides the capacity to predict a differentiation cascade based on precise synchronization of the stem cell cycle in the presence of an appropriate inducing stimulus. [Quesenberry Declaration, par. 8; all emphasis added.]

By way of example, Dr. Quesenberry states:

18. In the present application, the data showed that Lineage^{negative} Rhodamine^{low} Hoescht^{low} ("LRH") cells synchronized and then sub-cultured separately inductive differentiation cocktail (GM-CSF, G-CSF, and steel factor) prior to cell division showed marked variations in differentiated cell production with the first cell cycle transit (Example 1; Figures 1-4). Surprisingly, megakaryocyte differentiation and proliferative granulocyte differentiation were amplified at G₀ to mid-S phase [defined in the specification as **32-hour differentiation hotspot**], whereas non-proliferative granulocyte differentiation was amplified at G₀ to late S phase [defined in the specification as **40-hour differentiation hotspot**] (Example 1; Figures 1-6).

19. The results are surprising, because these differences provide evidence for a flexible system for hematopoietic regulation in which multiple different outcomes can occur dependent on cell cycle phase and specific microenvironmental influences as part of a *continuum* of *reversible* phenotypic shifts (*in contrast to a hierarchical model*) with continuous change in a *reversible* fashion.

20. None of the references cited by the Patent Office addresses this concept or teaches or suggests this concept. [Quesenberry Declaration, pars. 18-20; all emphasis added; all bracketed material added.]

Clearly, Applicant is not "merely giving a name to a phenomenon that occurs during the claimed method" (Office Action, p. 7), especially where Applicant has provided methods

favoring differentiation of bone marrow stem cells into diverse hematopoietic cell types based on selectively triggering a particular differentiation cascade as a function of cell cycle phase of a synchronous population of stem cells.

In view of the foregoing, Applicant respectfully disagrees with the Patent Office's position, because taken in the context of this passage and other passages in the specification (including the Examples), Applicant submits that one of ordinary skill in the art would readily comprehend the claim language of "reversible differentiation hotspot." Applicant has amended claim 62 to reiterate the term "reversible," however.

In the event that this issue is the only remaining issue in the prosecution of the application, however, Applicant would be willing to work with the Patent Office to amend the claims to place them in condition for allowance.

F. Claims 64, 65, and 72

The Patent Office finally rejected dependent claims 64, 65, and 72, which depend from independent claims 54, 1, and 68, respectively, alleging that the term "the bone marrow stem cells" in reference to the respective independent claims, is confusing, because each of the independent claims refers to several different versions of BMSCs (Final Office Action, p. 7; *but see* MPEP §2173.05(e)).

Although the rejection does not specify, Applicant is assuming that the Patent Office means to make a rejection based on an alleged lack of antecedent basis for the term "the bone marrow stem cells." The specific reference to "bone marrow stem cells" in each of the underlying independent claims (claims 1, 54, and 68) is in step (a) of each claim. Applicant

respectfully disagrees with the Patent Office's position, because Applicant submits that one of ordinary skill in the art would readily comprehend the antecedent basis for the dependent claim language of "the bone marrow stem cells."

However, claims 64, 65, and 72 have been canceled without prejudice, and the rejection of these claims is thereby rendered moot.

Applicant respectfully submits that remaining claims 54 and 57-62 fulfill the requirements of 35 U.S.C. §112, second paragraph, thereby placing these claims in condition for allowance, and requests the Examiner's reconsideration accordingly. In the event that this issue is the only remaining issue in the prosecution of the application, however, Applicant would be willing to work with the Patent Office to amend the claims to place them in condition for allowance.

V. The Rejection of Claims 1-4, 6-9, 11-13, 29-30, 54-62, and 64-81 Under 35 U.S.C. §102(b) over Hagihara is Traversed in Part and Rendered Moot in Part

The Examiner has finally rejected claims 1-4, 6-9, 11-13, 29-30, 54-62, and 64-81 under 35 U.S.C. §102 for alleged anticipation by Hagihara et al. (J. Immunol. Methods 253: 45-55 [2001]). Applicant traverses the rejection and respectfully requests reconsideration of these claims.

Claims 1-4, 6-9, 11-13, 29-30, 55-56, 64-66, and 68-81 have been canceled without prejudice, and the rejection is rendered moot with respect to these claims.

Under 35 U.S.C. §102(b):

A person shall be entitled to a patent unless –

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of the application for patent in the United States,...

To anticipate a claim, a single prior art reference must teach each and every element of the claim (*Verdegaal Brs. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 [Fed. Cir. 1987]; MPEP §2131). “Every element of the claimed invention must be literally present, arranged as in the claim....The identical invention must be shown in as complete detail as is contained in the patent claim....” (*Richardson v. Suzuki Motor Co.*, 868 F.2d 1226, 1236, 9 USPQ2d 1913, 1920 [Fed. Cir. 1989] [citations omitted]; MPEP §2131).

The Patent Office alleges:

In this case, even if applicant had identified some previously unappreciated properties of Hagihara’s method steps (which the examiner does not concede), the steps themselves would not become patentable. See *Titanium Metals Corp. v. Banner*, 778 F.2d 775, 227 USPQ 773 (Fed. Cir. 1985) [hereinafter “*Titanium Metals Corp.*”]. Hagihara teaches steps that overlap with the instantly claimed steps, and the examiner notes that Hagihara’s cocktail (Flt-3, SCF, and TPO) is identical to that employed by applicants in the working examples. See specification, page 10, line 16, e.g.; the molecule called “steel factor” is also known as “stem cell factor” or SCF [citation omitted]. The examiner also notes that this combination of factors was known in the art at the time of filing to synchronize the cell cycle of cultured BMSCs....[Final Office Action, pp. 8-9; citations omitted; italics in original; other emphasis added.]

Applicant respectfully disagrees and traverses this rejection, both for the reasons already of record and additionally here.

The present situation is distinguishable from *Titanium Metals Corp.* In *Titanium Metals Corp.*, the patent claims were directed to compositions (titanium base alloys), and the prior art reference disclosed compositions (titanium base alloys) (*Titanium Metals Corp. v.*

Banner, 778 F.2d at 776; *see also Akzo N.V. v. U.S. Int'l Trade Comm'n*, 808 F.2d 1471, 1479, 1 USPQ2d 1241 [Fed. Cir. 1986]). The applicant attempted to argue that the properties of its claimed alloys were not disclosed in the prior art reference, although the alloys themselves were (*Id.* at 777).

In contrast, the claimed invention is directed to methods having limitations as defined in the claims. Previously in step b) of independent claim 54, the cells of step a) were contacted with at least one growth factor or cytokine “at a predetermined phase of the cell cycle” and then subcultured in step c) to produce differentiated hematopoietic cells dependent on whether the “predetermined phase of the cell cycle” is “mid-S phase” or “late S phase” (all emphasis in quotations added).

However, in Hagihara, the description of the method (p. 49) makes no reference to any particular cell cycle phase or even to the cell cycle as a whole. Applicant respectfully submits that Hagihara fails to provide an inherent or enabling anticipation of either the previous version of claim 54 or the present amended version of claim 54.

It appears that the Patent Office is attempting to argue that (i) the method described in Hagihara would result in synchronous cells; (ii) treatment of the synchronous cells, the timing of which is not clearly defined by Hagihara, might randomly occur at a particular point in the cell cycle claimed by the Applicant; and (iii) differentiated cells would be produced. The Patent Office attempts to bolster the second point by arguing that the culture of Hagihara could inherently include cells at an appropriate stage of the cell cycle at the time of exposure to a growth factor or cytokine.

This assertion ignores the fact that the claims are directed to specific or pre-determined phases of the cell cycle as express claim limitations – not simply an “unstated limitation” in Hagihara, in which there was no limitation at all.

Hagihara describes a method of *ex vivo* production of dendritic cells from CD34 positive umbilical cord blood or bone marrow cells using mouse stromal cells and a HESS-5 culture system with three cytokines (Flk-2/Flt-3 ligand, stem cell factor, and thrombopoietin) to produce primitive myeloid cells which can develop into dendritic cells. A “[l]ong-term” liquid culture of CD34+ cells is carried out, and the expanded cells are “divided into two parts every week...” (Hagihara, p. 49; all emphasis in quotations added). One part is used for continued expansion, while the other part is checked for phenotypes (CD34/38, CD14/33) and used for dendritic cell induction for a period of 0-14 days (Hagihara, p. 49).

As noted by Dr. Peter Quesenberry:

....**Hagihara...does not disclose** contacting synchronously cycling cells with a growth factor or cytokine “at a predetermined phase of the cell cycle.” Instead, the cells in **Hagihara** were subsequently subcultured “every week” with no disclosure regarding the timing of the subculturing with regard to the cell cycle. Hagihara does not even disclose whether the subculturing of the cells took place at the same time of day on the same day of “every week.” the [sic] claims are limited by the phase of the cell cycle, which **Hagihara is not**. Rather, **Hagihara** discloses a vague, imprecise, and random period of time (“every week” – without even any discussion of the day, time, or duration of cell cycle), unlike the Applicants, whose claims are directed to a specific phase of the cell cycle (“predetermined phase of the cycle”), regardless of when in time that phase occurs. In essence, Applicants are claiming a method based on cell cycle, while Hagihara discloses a method based on time. Even if it were possible to know the duration of the cell cycle in numbers of hours (and it is not), Hagihara’s vague, imprecise, and random “every week” neither discloses nor suggests a consistently specific cell cycle phase. Therefore, that it cannot be concluded that Hagihara subcultured synchronously cycled cell cultures with a growth factor or cytokine “at a predetermined phase of the cell cycle”....[Quesenberry Declaration, par. 8; all emphasis added.]

More recently, with respect to at least some of the claims (unspecified), the Patent Office argues:

[T]he claims do not require that the contacting commence at any particular point in the cell cycle, only that cells be contacted at a particular point. A method like Hagihara’s, which teaches continuous contact over 14 days, includes contact 32 and 40 hours from the start of culturing. [Final Office Action, pp. 10-11.]

(Applicant wishes to note parenthetically that the Patent Office seems to have misunderstood Applicant's earlier argument concerning the synchronous cell cultures of step a) and that Applicant was not attempting to provide a new and different meaning for the word "comprising" [p. 11.] Rather, Applicant was merely pointing out that synchronous cells are synchronous by definition.)

The Patent Office further alleges:

The Quesenberry declaration argues that Hagihara teaches culturing for particular times, while the instant method is "directed to a specific phase of the cell cycle,...regardless of when in that time that phase occurs." See page 8, near the center of the page. The examiner queries the difference as far as it is relevant to the properties of the positively recited method steps, since Figure 3 of Colvin 2010 clearly teaches culturing BMSCs in SCF/SF, Flt-3, and TPO for various times. [Final Office Action, p. 12.]

With respect to the Patent Office's remarks (pp. 11-12) concerning the Quesenberry Declaration and concerning Colvin et al. ("Heterogeneity of Non-Cycling and Cycling Synchronized Murine Hematopoietic Stem/Progenitor Cells," J. Cell. Physiol. 222:57-65 [2010] [hereinafter "Colvin 2010"]), essentially, Applicant's methods include a step of culturing bone marrow stem cells under the disclosed conditions until they are synchronous, then stimulating the synchronous cells with the disclosed growth factors/cytokines at a specific or pre-determined phase of the cell cycle, and then subculturing them until differentiated hematopoietic cells of cell-cycle specific types are produced. Applicant had introduced Colvin 2010 as evidence of post-filing support of the methods of the present invention (see e.g., Figure 6 of Colvin 2010).

The mere fact that Hagihara's cells might be cycling synchronously and just might happen to be contacted by the appropriate growth factor or cytokine at a given time in the cell cycle and just might produce the appropriate "cell cycle-specifically differentiated hematopoietic cells" does not provide an enabling anticipation – even inherently – of

contact at a **“specific” or “predetermined” phase of the cell cycle or at “mid-S phase”**.

Moreover, if the initially cultured cells were to be contacted during the “wrong” phase,” one of ordinary skill in the art would expect that Hagihara’s method might **not** work to produce the desired cell cycle-specifically differentiated hematopoietic cells resulting from the methods of the claimed invention; certainly, **there is nothing to suggest** that Hagihara’s method would produce the appropriate cell cycle-specifically differentiated hematopoietic cells **automatically**, and **no evidence has been cited by the Patent Office** to show that it would.

In addition to traversal of the rejection to claim 54 in its previous form, Applicant has amended claim 54. As currently amended, step a) of claim 54 provides for the initial culturing of LRH bone marrow stem cells in “a combination of steel factor, thrombopoietin, and FLT3-ligand;” step b) of claim 54 specifies that the resultant “synchronously progressing bone marrow stem cells of step a)” are brought into contact with “a combination of G-CSF, GM-CSF, and steel factor commencing at mid-S phase of the cell cycle;” and step c) of claim 54 specifies the comparatively greater numbers of resultant differentiated hematopoietic cells (megakaryocytes, platelets, proliferative granulocytes) produced after subsequent subculturing.

In addition to the failure of Hagihara to make any reference to any particular cell cycle phase or even to the cell cycle as a whole, Hagihara also fails to disclose the use of LRH cells; the initial culturing of the LRH cells in “a combination of steel factor, thrombopoietin, and FLT3-ligand;” the synchronous progression of the cultured cells; the contacting of the synchronous cells with “a combination of G-CSF, GM-CSF, and steel factor commencing at mid-S phase of the cell cycle;” or the comparatively greater numbers of resultant differentiated hematopoietic cells (megakaryocytes, platelets, proliferative granulocytes) produced after subsequent subculturing.

Claims 57-58, 60-62, and 67 are dependent on claim 54 and the same arguments apply to these claims as well.

Claim 59 was previously dependent on underlying claim 54, which previously contained language directed in the alternative to mid-S phase or to late S phase. Claim 54 was amended to be directed to methods in which contact of the synchronous cells commenced at mid-S phase. New independent claim 84 is similarly directed to methods in which contact of the synchronous cells commences at late S phase. As a result, claim 59, which is directed to the timing of late S phase, has been amended to be dependent on underlying new claim 84.

With respect to underlying new claim 84, step a) provides for the initial culturing of LRH bone marrow stem cells in “a combination of steel factor, thrombopoietin, and FLT3-ligand;” step b) specifies that the resultant “synchronously progressing bone marrow stem cells of step a)” are brought into contact with “a combination of G-CSF, GM-CSF, and steel factor commencing at late S phase of the cell cycle;” and step c) specifies the comparatively greater numbers of resultant differentiated hematopoietic cells (mature or non-proliferative granulocytes) produced after subsequent subculturing.

In addition to the failure of Hagihara to make any reference to any particular cell cycle phase or even to the cell cycle as a whole, Hagihara also fails to disclose the use of LRH cells; the initial culturing of the LRH cells in “a combination of steel factor, thrombopoietin, and FLT3-ligand;” the synchronous progression of the cultured cells; the contacting of the synchronous cells with “a combination of G-CSF, GM-CSF, and steel factor commencing at late S phase of the cell cycle;” or the comparatively greater numbers of resultant differentiated hematopoietic cells (mature or non-proliferative granulocytes) produced after subsequent subculturing.

Again, the mere fact that Hagihara's cells might be cycling synchronously and might just happen to be contacted by the appropriate growth factor or cytokine at a given time in the cell cycle does not provide enabling anticipation – even inherently – of contact specifically “in mid-S phase” (or “in late S phase” in the case of now-amended claim 59) of the cell cycle. Hagihara's method on page 49 not only fails to mention “mid-S phase” (or “late S phase”), let alone any or all phases of the cell cycle, but Hagihara's vague reference to subculturing “every week” actually implies the reverse – namely, that the particular cell cycle phase at a given time is unimportant, thereby teaching away from the present invention (all emphasis in quotations added). Again, it is not simply a question of an “unstated limitation” in Hagihara. Rather, there is no real limitation in Hagihara at all, and these elements of the claims are absent, and not even implied, in the disclosure of Hagihara.

Clearly, a disclosure, whether express or implied, that is lacking in the first instance cannot possibly be considered an enabling anticipatory disclosure to one of ordinary skill in the art.

Therefore, Hagihara fails to provide an anticipatory disclosure, either express or implied, with respect to remaining claims 54, 57-62, and 67.

However, the Patent Office made additional remarks with respect to some of the dependent claims, as well as with respect to some of the independent claims.

A. Claims 4, 54, 57, and 59

The Patent Office alleges that claims 4, 54, 57, and 59 do not clearly limit the claims, “because they appear merely to recited properties of the cell cycle of synchronized BMSCs” (Final Office Action, p. 9). Claim 4 is canceled without prejudice, and the rejection is moot with respect to this claim. Claims 57 and 59 are dependent on underlying independent claim 54.

1. Claim 57

Claim 57 is directed to the methods of underlying claim 54, wherein the subculturing step “is carried out for about 14 days.” Applicant traverses this rejection and respectfully submits that the Patent Office’s remarks do not appear to relate to the limitations of these claims and that the time limitations for the subculturing steps clearly do not recite a property of the cell cycle.

2. Claim 54

Applicant also traverses the rejection of independent claim 54, which has a complex series of method steps, as discussed at length above, and **does not merely recite properties of the cell cycle of BMSCs**. For example, the practitioner of the claimed method of claim 54 would need to a) culture the BMSCs (now amended to be LRH cells) under conditions that promote synchronous progression through the cell cycle; b) contact the synchronous cells with at least one growth factor or cytokine at a predetermined phase of the cell cycle (now “mid-S phase”); and c) subculture the cells until differentiated hematopoietic cells of the types recited in the claim are produced.

3. Claim 59

Applicant also traverses the rejection of claim 59, which further defines the timing of late S phase and which is now dependent on new underlying claim 84, which is directed to S phase.

B. Claims 13, 60, 69, 75, and 79

The Patent Office alleges that Hagihara’s method includes separating the cells induced to become dendritic cells and this also anticipates the isolating step claims 13, 60, 69, 75, and

79. Claims 13, 69, 75, and 79 have been canceled without prejudice, and the rejections of these claims are rendered moot. However, claim 60 is dependent on underlying claim 54, and Applicant respectfully traverses the rejection of claim 60 for the reasons discussed *supra* for underlying independent claim 54.

C. Claims 7-9, 12, and 54

The Patent Office alleges further that Hagihara's method inherently includes the cell types recited in claims 7-9, 12, and 54. Claims 7-9 and 12 have been canceled without prejudice, and the rejection is moot with respect to these claims. However, Applicant respectfully traverses the rejection of claim 54 for the reasons discussed *supra*.

The Patent Office states that "[i]f applicants' method differs materially from Hagihara's, e.g. by amounts of growth factors or timing of culture steps, the claims should so recite" (Final Office Action, p. 9). Applicant respectfully submits that claim 54 already previously contained limitations concerning the "timing" of step b) with reference to **the given phase of the cell cycle**. Previously, claim 54 was directed either to mid-S phase or to late S phase. As amended, it is now directed to mid-S phase.

D. Claims 29, 30, 61, 62, 70, 71, 76, 77, 80, and 81

As discussed *supra*, the Patent Office had rejected these claims for alleged indefiniteness under 35 U.S.C. §112, second paragraph. With reference to this rejection, the Patent Office further alleges:

[C]laims 29, 30, 61, 62, 70, 71, 76, 77, 80, and 81 appear merely to give a name to a phenomenon that occurs during the positively recited culturing steps of the claims, so they do not clearly limit the claims. Applicants' disclosure appears to be an investigation of a mechanism in which particular points in the cell cycle are more conducive to yielding one endpoint over another. However, [p]atents are not awarded for academic theories, no matter how groundbreaking or necessary to the later patentable inventions of others." *Ariad Pharms. Co. v. Eli Lilly & Co.*, 94 USPQ2d 1161, 1173 (Fed. Cir. 2010) (en banc). The patent system is designed to give incentives to complete

inventions, not to guess at the future. *Id.*, at 1174. Methods are defined by their steps, not by any underlying mechanism. The fact that Hagihara did not recognize the presence of a “hotspot” within their culturing steps cannot distinguish that reference from the claimed method as long as the positively recited steps are taught by the reference. [Final Office Action, pp. 9-10; italics in original.]

Claims 29-30, 70-71, 76-77, and 80-81 have been canceled without prejudice, and the rejection is moot with respect to these claims. However, claims 61 and 62 remain in the application in amended form.

Applicant respectfully traverses this rejection. First, the present invention is not a mere academic theory. The claimed invention provides practical methods via actual reduction to practice for making use of the inventor’s discovery in order to produce from bone marrow stem cells populations of differentiated hematopoietic cells having enhanced percentages of desired cell types. Second, these methods involve a series of steps outlined in the claims. In contrast to the Patent Office’s assertion that “[m]ethods are defined by their steps” (Final Office Action, p. 10), Hagihara fails to recite the method steps of the claims, as discussed *supra*.

E. Claims 64, 65, and 72

The Patent Office alleges further that “the amendments to claims 1, 54, and 68 introduce uncertainty into the limitations of claims 65, 64, and 72, which depend from them and refer to some population of BMSCs within those claims” and that “[c]laims 64, 65, and 72 do not clearly limit the independent claims” (Final Office Action, p. 10; all emphasis added).

Claims 65 and 72 and their underlying claims 1 and 68 have been canceled without prejudice, and the rejection is moot with respect to these claims. Claim 64 has been canceled without prejudice, and the rejection is moot with respect to this claim. Underlying claim 54 has been amended to include the limitations of canceled claim 64.

Applicant has already traversed the rejection of the remaining underlying independent claim 54 with respect to the alleged uncertainty of these claims (see comments *supra* with respect to the rejections of these claims under 35 U.S.C. §112, second paragraph). Applicant has introduced the limitations of claim 64 into independent claim 54, which **further limits the bone marrow stem cells to bone marrow stem cells comprising Lineage^{negative}Rhodamine^{low}Hoescht^{low} (LRH) cells.** The Patent Office has failed to show that these cells are identical to the cells of Hagihara.

Applicant respectfully submits that remaining claims 54 and 57-62 fulfill the requirements of 35 U.S.C. §102(b), thereby placing these claims in condition for allowance, and requests the Examiner's reconsideration accordingly.

VI. The Rejection of Claims 1-4, 6, 11, 13, 29, 30, 64, 65, 68-72, and 74-81 under 35 U.S.C. §103(a) over Hagihara Taken with Yan and Messner is Traversed, but Rendered Moot

The Examiner has rejected claims 1-4, 6, 11, 13, 29, 30, 64, 65, 68-72, and 74-81 under 35 U.S.C. 103(a) as unpatentable over Hagihara et al. (J. Immunol. Methods 253: 45-55 [2001]) in view of Yan et al. (Blood, 96(11; part 1): 680a (November 2000) ("Yan")); and Messner et al. (Blood 70(5): 1425-1432 (November 1987) ("Messner")). Applicant traverses the rejection, but the rejection is rendered moot.

Claims 1-4, 6, 11, 13, 29, 30, 64, 65, 68-72, and 74-81 have been canceled without prejudice. Therefore, the rejection is rendered moot with respect to these claims is technically moot.

VII. The Rejection of Claims 7-9, 54-62, and 64, and Possibly Claim 65, under 35 U.S.C. §103(a) over Hagihara, Yan, and Messner and Further in View of Klabusay and Ramsfjell is Traversed, but Rendered Moot in Part

The Examiner has rejected claims 7-9, 54-62, and 64, and possibly claim 65, under 35 U.S.C. 103(a) as unpatentable over Hagihara et al. (J. Immunol. Methods 253: 45-55 [2001]), Yan et al. (Blood, 96(11; part 1): 680a (November 2000) ("Yan")), and Messner et al. (Blood 70(5): 1425-1432 (November 1987) ("Messner")), as applied to claims 1-6, 11, 13, 29, 30, 65, 68-72, and 74-81, and further in view of Klabusay et al. (2002, *Blood* 100: Abstract No. 4118; hereinafter "Klabusay") and Ramsfjell et al. (1996, *Blood* 88: 4481-4492; hereinafter "Ramsfjell"). Applicant traverses the rejection and respectfully request reconsideration of these claims.

Claims 7-9, 55-56, and 64-65 have been cancelled without prejudice. The rejection is moot with respect to these claims.

Although all claims 1-4, 6, 11, 13, 29, 30, 64, 65, 68-72, and 74-81 (see VI, *supra*), which were rejected under 35 U.S.C. §103(a) over Hagihara in view of Yan and Messner, were canceled, thereby rendering that rejection moot, the Patent Office refers to those arguments with respect to the present rejection, so it is necessary to refer in this section (section VII) to those arguments made in the Office Action with respect to previous section VI and to reply to the same before addressing the remaining remarks for the present section.

With respect to the rejection of the claims in section VI, the Patent Office alleged, in pertinent part:

....Hagihara does not indicate that the growth factor must be added during any particular cell cycle phase (although, as discussed above, the claims also make no such requirement; see M.P.E.P. § 2111.03).

Yan teaches that the combination of factors SCF, TPO and FLT-3 in the culture medium stimulates the hematopoietic bone marrow cells to enter into synchronous cell cycle from resting state. For example, see the abstract of Yan, which clearly discloses that the purified bone marrow cells were quiescent (non-dividing or “resting” at G0/G1 phase) at the beginning of the culture, that the addition of cytokines SCF, TPO and FLT-3 stimulated the cells to enter into the cycle, and that the amount of synchronous cells in S phase increased during culturing in the presence of cytokines SCF, TPO and FLT-3.

Messner teaches that cell cycle studies and stem cell engraftment studies indicate that the higher than normal proportions of multipotential hematopoietic cells are present in S phase during progression of the hematopoietic cells through the cell cycles. See abstract, e.g.

A person of ordinary skill in the art would have had a reasonable expectation of success in synchronizing the BMSCs of Hagihara using Hagihara’s medium containing SCF, TPO, and FLT-3 ligand because Yan teaches that such a medium promotes synchronous progression through the cell cycle. The skilled artisan would have been motivated to synchronize the cells in order to obtain more consistent results from the culturing step, **especially given Messner’s teaching that the cell cycle phase affects the proportion of multipotential cells in a population.**

The skilled artisan would have had a further reasonable expectation of success in synchronizing the cells and adding growth factor or cytokine (in this case, the GM-CSF of Hagihara) at various phases of the cell cycle because **Messner teaches that more hematopoietic stem cells are at S phase** than other cell cycle phases. The skilled artisan would have been motivated to determine the differentiability of Hagihara’s stem cells at various points in the cell cycle in order to maximize the number of stem cells available for Hagihara’s differentiation protocol. “When there is a design need or market pressure to solve a problem and there are a finite number of identified, predictable solutions, a person of ordinary skill has good reason to pursue the known options within his or her technical grasp. If this leads to the anticipated success, it is likely the product not of innovation but of ordinary skill and common sense. In that instance the fact that a combination was obvious to try might show that it was obvious under §103.” *KSR International Co. v. Teleflex Inc.*, 82 USPQ2d 1385, 1397 (U.S. 2007). In this case, there are only a few different points in the cell cycle, and Messner teaches that these points were well known at the time of the invention; **testing stem cells at each of these points to identify their propensity for differentiation would have constituted routine experimentation at the time of the invention.**

It would therefore have been obvious to a person of ordinary skill in the art at the time the invention was made to synchronize the cells of Hagihara with the medium of Hagihara and Yan and then to treat the synchronized cells at

various points within the cell cycle in order to determine the optimal conditions for Hagihara's differentiation method. [Final Office Action, pp. 14-16; italics in original; all other emphasis added.]

Applicant disagrees and respectfully traverses this rejection. Hagihara has been discussed at length, *supra*, with respect to alleged anticipation, and the same arguments apply here.

Also with respect to the rejection of section VI, the Patent Office further alleged:

Regarding the pertinent rejection of record, applicant alleges that the skilled artisan would have assumed that BMSCs would be homogeneous and clonal in nature, referring generally to the Quesenberry declaration. See reply, page 27....

There is no evidentiary basis for the Quesenberry declaration's characterization of the prevailing assumptions in the art about BMSCs. As discussed previously, that declaration contains only the opinion of the inventor himself, and evidence is necessary to overcome the examiner's *prima facie* obviousness rejection, which was based on art teachings. Applicant refers to an unidentified portion of the Quesenberry declaration that presumably alleges that the skilled artisan would have presumed that a population of BMSCs would be "homogeneous and clonal in nature," but the Colvin 2010 publication, which application submitted with the instant reply to supplement the Quesenberry declaration, characterizes CFU-S, which are immature hematopoietic stem cells derived from bone marrow, as "heterogeneous." See page 57, column 1. The Colvin 2010 publication refers to a 1964 study that concluded that CFU-S are heterogeneous and unpredictable, and it further discusses studies from as early as 1987 tending to show that CFU-S are heterogeneous. See *id.* Regarding the LRH cells of the Quesenberry declaration and the instant specification, Colvin 2010 refers to 1 1991 publication and indicates that "[o]ne in 3-4 of these cells will form high-proliferative potential (HPP) colonies," which suggests that the art never considered LRH to be "homogeneous and clonal in nature." The examiner queries this seeming inconsistency. [Final Office Action, pp. 16-17; italics in original.]

First, Applicant respectfully points to **paragraph 3 of the Quesenberry Declaration, which outlines the hierarchical model of stem cell regulation (as distinct from the clonal succession model)**:

3. **In general, models of stem cell regulation have been hierarchical. In these models, a primitive stem cell having great potential give [sic] rise to a proliferating progenitor pool, which in turn gives rise to recognizable differentiated cells.** During this process, **proliferative potential is lost**, while **specific differentiated features are acquired**. Presumptively, but without definite proof, there is also self-renewal at the most primitive stem cell level, and this self-renewal is also lost with differentiation. **Many data exist to support such a hierarchical model.** Marrow cells have been separated with short- and long-term repopulation potential, and progenitors have been characterized as exclusively committed to the production of restricted progeny. In addition, the clear expansion of different progenitor types in cytokine-stimulated in vitro culture with a loss of long-term engraftment capacity speaks to the existence of a progenitor hierarchy, at least at the more differentiated levels. Not all data fit this model, however. It has been observed, for instance, that “daughter cell” or paired-progenitor experiments indicate that a primitive progenitor spleen cell can make totally different lineage choices during one cell cycle transit, such that, for example, one daughter cell forms one type of cell colony, while a different daughter cell forms a different type of cell colony. **An intrinsic component of the hierarchical model is that the most primitive hematopoietic stem cell is a quiescent cell in G₀ having a fount of potential without differentiated characteristics.** It has generally been believed that primitive hematopoietic stem cells were dormant or quiescent and were thus protected from depletion or exhaustion. [Quesenberry Declaration, par. 3, p. 6; all emphasis added.]

Second, Colvin 2010 (p. 57, col. 1) compares the work on CFU-S (a different cell line from the LRH used in the present specification and in Colvin 2010), which are described in Colvin 2010 as “a heterogeneous population of stem cells” **followed by the observation:**

...In contrast, **many investigators have put forward single cell clonal engraftment as the gold standard** for defining a hematopoietic stem cell. **This latter, of course, implies homogeneity of the stem cell populations.** [Colvin 2010, p. 57, col. 1; all emphasis added.]

Additionally with respect to the rejection of section VI, the Patent Office further alleged:

In response to applicant’s argument that Messner was concerned with a different aspect of BMSC cell cycling, the fact that applicant has recognized another advantage which would flow naturally from following the suggestion of the prior art cannot be the basis for patentability when the differences would otherwise be obvious. See *Ex parte Obiaya*, 227 USPQ 58, 60 (Bd. Pat. App. & Inter. 1985). Yan teaches that synchronizing BMSCs was known in the art, and Messner’s teachings suggest that the multipotentiality of BMSCs varies during the cell cycle. When Hagihara’s teaching of culturing BMSCs first in a cocktail that Yan teaches synchronizes cells,

then in a differentiation-inducing culture media, is considered with Messner's suggestion that cell cycle affects multipotentiality, the invention becomes obvious. The examiner emphasizes again for the record that the claims do not require adding growth factors at any particular time, only that BMSCs be in contact with the factors at a given point. [Final Office Action, p. 17; italics in original.]

Applicant respectfully submits that this reasoning overstates the teachings both of Hagihara and of Messner.

With respect to the present rejection in this section (section VII), the Patent Office incorporates the above arguments and alleges in pertinent part:

Hagihara does not teach culturing in G-CSF. Hagihara does not teach all of the end points in claims 7-9, 12, and 54. Hagihara does not discuss the markers in claim 64.

Klabusay teaches that hematopoietic stem cells are able to regenerate hematopoiesis in all lineages and that addition of G-CSF to their medium will significantly increase the number of matured cells including granulocytes. See abstract, e.g.

Ramsfjell teaches that culturing stem cells in SCF enhances megakaryocyte differentiation, as well as production of granulocytes and other mature hematopoietic cell types. See abstract, e.g. Ramsfjell teaches that when megakaryocytes mature, they produce platelets. *Id.*

A person of ordinary skill in the art would have had a reasonable expectation of success in substituting the G-CSF of Klabusay or the SCF of Ramsfjell for the GM-CSF of Hagihara in Hagihara's method taken in view of Yan and Messner because Klabusay and Ramsfjell teach that G-CSF and SCF affect the differentiation of Hagihara's cells. The skilled artisan would have been motivated to make such a substitution to determine whether Hagihara's method can be used with Klabusay's and Ramsfjell's growth factors/cytokines to direct differentiation to the endpoints already associated by Klabusay and Ramsfjell with those growth factors/cytokines. Varying Hagihara's method using these two different growth factors/cytokines and assaying for directed differentiation to the limited outcomes taught by Klabusay and Ramsfjell would have constituted routine experimentation at the time of the invention. See *KSR* at 1397.

It would therefore have been obvious to a person of ordinary skill in the art at the time the invention was made to vary the growth factor/cytokine in Hagihara's differentiation method in order to identify the effects of such

variance on that method because Klabusay and Ramsfjell identified links between various growth factors and particular differentiation endpoints.

Therefore, the invention as a whole would have been *prima facie* obvious to a person of ordinary skill at the time the invention was made. [Final Office Action, pp. 18-19; italics in original; other emphasis added.]

Hagihara has been discussed at length *supra* in connection with the rejection under 35 U.S.C. §102(b), in the Quesenberry Declaration, and throughout prosecution, and the same reasoning applies here.

In brief, **the present claims require** that the cells in step b) be contacted commencing in **“mid-S phase”** (claim 54) (or **“late S phase”** [amended claim 59 now dependent on new underlying claim 84; previous alternative limitation in claim 54]) and **also require** that the contacted cells be subcultured to **produce “differentiated hematopoietic cells”** (claim 54) with comparatively increased numbers of “megakaryocytes,” “platelets,” or “proliferative” granulocytes (or “mature or non-proliferative granulocytes” in claim 59 due to limitations in new underlying claim 84; previous alternative limitation in claim 54)) (all emphasis added).

Hagihara fails to disclose or suggest, either expressly or inherently, the claimed methods. The method of **Hagihara (p. 49) never refers to the cell cycle, much less the different stages of the cell cycle.** **Hagihara has no reference to “mid-S phase” or “late S phase,”** and **Hagihara’s vague reference to subculturing “every week” actually implies the reverse – namely, that the particular cell cycle phase at a given time is unimportant, thereby teaching away from the present invention** (all emphasis in quotations added). In addition, **Hagihara makes no mention or suggestion of variations in resulting cell populations, much less any connection between phases of the cell cycle and the resulting differentiated hematopoietic cells.** It is *not* simply a question of an express disclosure, an “unstated limitation,” or a suggestion in Hagihara. Rather, **there is no real limitation in Hagihara at all, and these elements of claim 54 and the other remaining claims are absent and certainly neither implied nor suggested.**

In addition to the failure of Hagihara to make any reference to any particular cell cycle phase or even to the cell cycle as a whole or the failure of Hagihara to disclose or suggest the comparatively greater numbers of specific resultant cell types of the present invention, Hagihara also fails to disclose the use of LRH cells; the initial culturing of the LRH cells in “a combination of steel factor, thrombopoietin, and FLT3-ligand;” the synchronous progression of the cultured cells; or the contacting of the synchronous cells with “a combination of G-CSF, GM-CSF, and steel factor commencing at mid-S phase of the cell cycle.”

Yan, Messner, Klabusay, and/or Ramsfjell, taken either alone or in combination together, fail to supply the deficiencies of Hagihara.

As noted in the Quesenberry Declaration, however, **the cell cycle in Messner was addressed primarily to determine the quantitative proportion of clonogenic precursors in S-phase, and this work is irrelevant to the present invention,** as it found variations in frequencies of clonogenic precursors in the normal donor population, but also included marrow from leukemic patients, which cannot be equated with normal marrow. The cell cycle was addressed primarily to determine the **proportion of clonogenic precursors in S-phase** by preincubation with tritiated thymidine, rather than synchronizing the cell cycles or by exposure of a synchronous population of stem cells “at a predetermined phase of the cell cycle.” **Messner fails to describe or suggest the claimed method of selecting a phase in the cell cycle to yield cell cycle specific cells,** as discussed in the Quesenberry Declaration.

The Patent Office alleges that combining the (alleged inherently) synchronous cells of Hagihara using the cocktail of Yan and the factors of Klabusay and Ramsfjell at Messner’s S-phase to yield an increased overall number of clonogenic precursors would somehow arrive at the present invention.

An increase in clonogenic precursors between, e.g., G-phase vs. S-phase implies neither a difference in potentiality between, e.g., one part of S-phase vs. another part of S-phase, nor a difference in enhancing production of particular resultant cell types.

Applicant respectfully submits that there is **no suggestion of differences within S-phase** that would have prompted one of ordinary skill in the art to apply Messner to synchronized cells simply to increase the overall number of clonogenic precursors.

More particularly, **Messner fails to describe or suggest the claimed method** of utilizing a phase in the cell cycle to amplify differentiation of synchronous stem cells into cell cycle specific differentiated cell types, as discussed in the Quesenberry Declaration. Messner is simply concerned with the **proportion** of clonogenic precursors **as a whole in S-phase in general** – **not** with any connection between “specific” or “pre-determined” phases of the cell cycle and the **resulting types of “cell cycle-specifically” differentiated hematopoietic cells or hematopoietic cells having a “cell cycle-specifically differentiated hematopoietic cell type”**. The present invention is **not merely**, as stated by the Patent Office, a method for identifying the **propensity of differentiation** of stem cells at a given phase of the cell cycle. Rather, **it is qualitative, whereas Messner is merely quantitative.**

An increase in the overall number of clonogenic precursors between, e.g., G-phase vs. S-phase does not imply a difference in potentiality between, e.g., one part of S-phase vs. another part of S-phase nor a difference favoring particular resultant cell types.

Applicant also respectfully submits that there is **no suggestion of differences within S-phase** that would have prompted one of ordinary skill in the art to apply Messner to synchronized cells simply to increase the overall number of clonogenic precursors, and the disclosures of Yan, Klabusay, and/or Ramsfjell do not remedy these deficiencies.

With respect to Klabusay and Ramsfjell, Applicant respectfully submits that while these references may disclose the generation of various hematopoietic lineages, **neither of these references, either alone or in combination with each other or with Hagihara and/or Yan and/or Messner, discloses or suggests the present invention.** Although **Klabusay, like Messner, mentions S-phase** in general in a quantitative sense, **it does not describe or suggest differences, e.g., between mid-S phase and late S phase,** as recited in **independent claim 54 – let alone differences in the types of differentiated cells produced.**

Applicant respectfully draws attention to the Examination Guidelines Update: Developments in the Obviousness Inquiry After *KSR v. Teleflex* (Fed. Reg. 75[169]: 53643-53660 [Sept. 1, 2010] [hereinafter the “2010 Guidelines”]). **The Patent Office has failed to show** that the present invention has in any way combined prior art elements according to known methods in Hagihara to yield predictable results, or that this is a case of simple substitution of one known element for another to obtain predictable results, or the use or application of a known technique to improve a similar device or method in the same way. **The Patent Office has not shown** that the present invention is the result of known work in one field of endeavor prompting a variation for use in the same field or in a different one based on either design incentives or other market forces predictable to one of ordinary skill in the art. Notwithstanding the allegations of the Patent Office, **the Patent Office has not shown** that the present invention is the result of predictable variation in Hagihara or that it resulted from the choice from a finite number of identified, predictable solutions (e.g., cell cycle phases) having a reasonable expectation of success (e.g., “cell cycle-specifically differentiated hematopoietic cell type”), **nor has it shown that it would have been obvious to try with a reasonable expectation of success** (e.g., “cell cycle-specifically differentiated hematopoietic cell type”). Moreover, there is **no teaching, suggestion, or motivation** in Hagihara in view of Yan, Messner, Klabusay, and/or Ramsfjell that would have led one of ordinary skill in the art to modify Hagihara or Messner to yield that cell cycle-specific hematopoietic cells of the present invention. (*Id.*)

While obviousness can be established by combining or modifying the teachings of the prior art to produce the claimed invention where there is some teaching, suggestion or motivation to do so, “[t]he mere fact that references can be combined or modified does *not* render the resultant combination obvious *unless the results would have been predictable to one of ordinary skill in the art*” (MPEP §2143.01 (underline in original; other emphasis added); *see also KSR Int’l Co. v. Teleflex Inc.*, 550 U.S. 398, 127 S.Ct. 1727, 82 USPQ2d 1385, 1396 [2007]), and the mere statement that the claimed invention is within the capabilities of one of ordinary skill in the art does not suffice to establish obviousness (MPEP §2143.01; *see also KSR Int’l Co. v. Teleflex Inc.*, 82 USPQ2d at 1396). The prior art can be modified or combined to establish *prima facie* obviousness, but one of ordinary skill in the art would have to have had a reasonable expectation of success or at least some degree of predictability at the time the invention was made (*In re Merck & Co., Inc.*, 800 F.2d 1091, 231 USPQ 375 [Fed. Cir. 1986]; MPEP §2143.02).

With respect to MPEP §2143, the culturing/subculturing method of the present invention does not fall under the category of a simple substitution (*see, e.g., In re O’Farrell*, 853 F.2d 894, 7 USPQ2d 1673 [Fed. Cir. 1988]); it is not the result of predictable variation or the choice from a finite number of identified, predictable solutions having a reasonable expectation of success, nor would it have been obvious to try with a reasonable expectation of success (*see, e.g., Pfizer v. Apotex*, 480 F.3d 148, 82 USPQ2d 1321 [Fed. Cir. 2007]; *Ex parte Kubin*, 83 USPQ2d 1410 [Bd. Pat. App. & Inter. 2007]); nor was there any teaching, suggestion or motivation in Hagihara or in Messner, either alone or in combination with each other and Yan, Klabusay, and/or Ramsfjell, that would have led one of ordinary skill in the art to modify one or more of these references or to combine their teachings to result in the method of the present invention (MPEP §2143).

Hagihara, either alone or in combination with Yan, Messner, Klabusay, and/or Ramsfjell, would not teach or suggest the present invention.

As a result, **one of ordinary skill in the art would *not* have been motivated to combine Hagihara, Yan, Klabusay, Ramsfjell, and/or Messner to arrive at the present invention.** One of ordinary skill in the art would ***not*** have considered it “obvious to try” with ***any reasonable expectation of success.***

Claim 54 is an underlying claim for remaining claims 57-58 and 60-62, and the arguments here with respect to claim 54 also apply to these claims. As noted *supra*, claim 59 has been amended to be dependent on new underlying claim 84, which is directed to methods concerning late S phase, and analogous arguments to those here would apply to claim 59 with respect to late S phase.

Applicant respectfully submits that remaining claims 54 and 57-62 fulfill the requirements of 35 U.S.C. §103(a), thereby placing these claims in condition for allowance, and requests the Examiner's reconsideration accordingly.

IX. The Rejection of Claims 66, 67, and 72 under 35 U.S.C. §103(a) over Hagihara, Yan, and Messner and Further in View of McGlave is Traversed, But Rendered Moot in Part

In the Final Office Action, claims 66, 67, and 72 stand rejected under 35 U.S.C. §103(a), as allegedly obvious over Hagihara et al. (2001, *Journal of Immunological Methods* 253: 45-55; hereinafter “Hagihara”) in view of Feng Yan et al. (2000, *Blood* 96: 680a; hereinafter “Feng Yan” or “Yan”) and Messner et al. (1987, *Blood* 70: 1425-1432; hereinafter “Messner”) and further in view of McGlave et al. (1997, U.S. Patent 5,605,829; hereinafter “McGlave”). This rejection is traversed.

Claims 66 and 72 have been canceled without prejudice, and the rejection is rendered moot with respect to those claims.

The Patent Office alleges in pertinent part:

Hagihara, Yan, and Messner are relied upon as above. These references do not teach isolating BMSCs by fluorescence activated cell sorting (FACS).

McGlave teaches isolating pluripotent CD34+ BMSCs using FACS. See column 2, lines 8-25.

A person of ordinary skill in the art would have had a reasonable expectation of success in substituting the FACS isolation step of McGlave for the density centrifugation step of Hagihara because McGlave teaches that FACS isolates a pure population of CD34+ cells. The skilled artisan would have been motivated to make the substitution because Hagihara recognized CD34+ cells as the important ones obtained in their density gradient centrifugation step, so the skilled artisan would have wanted to conduct Hagihara's method with as pure a population of useful starting material cells as possible.

Therefore, the invention as a whole would have been prima facie obvious to a person of ordinary skill at the time the invention was made.

Applicant's comments have been fully considered, but they are not persuasive of error because they do not particularly address these claims other than to note their addition to the application. See page 17. [Final Office Action, pp. 21-22.]

Applicant respectfully traverses this rejection. Hagihara and Messner have been discussed at length above, and the same reasoning applies here.

As a result, **one of skill in the art would *not* have been motivated to treat stem cells at different phases of the cell cycle, because one of skill in the art would have had *no reasonable expectation* of any difference in the subsequent differentiation of cell types.**

Yan, Messner, and/or McGlave, taken either alone or in combination together, fail to supply the deficiencies of Hagihara.

Moreover, McGlave's use of FACS to isolate pluripotent CD34+ bone marrow stem cells would not supply the deficiencies of the combination of Hagihara, Yan, and Messner.

Hagihara, either alone or in combination with Yan, Messner, and/or McGlave, would *not* teach or suggest the present invention.

One of ordinary skill in the art would *not* have considered it "obvious to try" with *any reasonable expectation of success*.

As a result, **one of ordinary skill in the art would not be motivated to combine Hagihara, Yan, Messner, and McGlave to arrive at the present invention.**

Applicant notes that claim 54 is the underlying claim for claim 67, which recites the isolation by FACS, and that the arguments made in the previous sections with respect to claim 54 also apply to this claim.

Applicant also respectfully notes that this is the first citation of McGlave, so that the comments previously submitted concerning these claims would naturally not address the teachings of McGlave. Moreover, Applicant was under no obligation to address the alleged teachings of the previously cited Herzog reference, which post-dated the present application.

Applicant respectfully submits that remaining claim 67 fulfills the requirements of 35 U.S.C. §103(a), thereby placing this claim in condition for allowance, and request the Examiner's reconsideration accordingly.

X. Interview Requested

In order to expedite prosecution and allowance of this application, Applicants request a personal or telephonic interview with the Examiner. The Examiner is invited to contact the undersigned to schedule an interview at her earliest convenience.

CONCLUSION

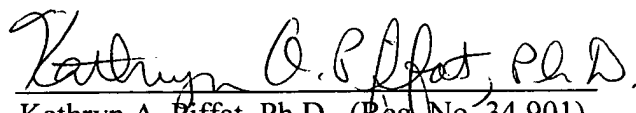
It is believed that all outstanding rejections have been addressed by this submission and that all the claims are in condition for allowance. If discussion of any amendment or remark made herein would advance this important case to allowance, the Examiner is invited to call the undersigned as soon as convenient.

In view of the foregoing amendments and remarks, the present application is respectfully considered in condition for allowance. An early reconsideration and notice of allowance are earnestly solicited.

Applicant hereby requests a three-month extension of time for the Amendment and accompanying materials. If, however, a petition for an additional extension of time is required, then the Examiner is requested to treat this as a conditional petition for an additional extension of time and the Commissioner is hereby authorized to charge our deposit account no. 04-1105 for the appropriate fee. Although it is not believed that any additional fee (in addition to the fee concurrently submitted) is required to consider this submission, the Commissioner is hereby authorized to charge our deposit account no. 04-1105 should any fee be deemed necessary.

Respectfully submitted,

Date: December 23, 2010


Kathryn A. Piffat, Ph.D., (Reg. No. 34,901)
Intellectual Property Practice Group
EDWARDS ANGELL PALMER & DODGE, LLP
P.O. Box 55874
Boston, Massachusetts 02205
Telephone: 617-239-0100

Customer No. 21874